Federal Democratic Republic of Ethiopia

EDICT OF GOVERNMENT

In order to promote public education and public safety, equal justice for all, a better informed citizenry, the rule of law, world trade and world peace, this legal document is hereby made available on a noncommercial basis, as it is the right of all humans to know and speak the laws that govern them.

ET ISO 13884 (2012) (English): Animal vegetables fats and oils-- Determination of isolated trans isomers by infrared spectrometry

ISO INSIDE

BLANK PAGE

BRUTUM FULMEN

IGNORANTIA JURIS NON EXCUSAT

PROTECTED BY COPYRIGHT

ETHIOPIAN STANDARD

ES ISO 13884:2012

First edition

Animal and vegetable fats and oils- Determination of isolated trans isomers by infrared spectrometry

(Identical with ISO 13884:2003)

ICS: 67.200.10

Published by Ethiopian Standards Agency
©ESA

Foreword

This Ethiopian Standard has been prepared under the direction of the Technical Committee for Animal and vegetables fats and oils (TC 23) and published by the Ethiopian Standards Agency (ESA).

The standard is identical with ISO 10540-2:2003, Animal and vegetable fats and oils -Determination of isolated trans isomers by infrared spectrometry, published by International Organization for Standardization (ISO).

For the purpose of this Ethiopian Standard, the adopted text shall be modified as follows.

- The phrase “International Standard” shall be read as “Ethiopian Standard”; and
- A full stop (.) shall substitute comma (,) as decimal marker.

Animal and vegetable fats and oils — Determination of isolated *trans* isomers by infrared spectrometry

1 Scope

This International Standard specifies a method for the accurate determination of isolated *trans* bonds in natural or processed long-chain fatty acids, fatty acid esters and triglycerides with *trans* isomer levels $\geqslant$ 5 %.

The method is not applicable, or is applicable only with specific precautions, to

- fats and oils containing high levels (over 5 %) of conjugated unsaturation (e.g. tung oil),
- materials containing functional groups which modify the intensity of the C-H deformation about the *trans* double bond [e.g. castor oil containing ricinoleic acid or its geometrical isomer, ricinelaidic acid (12-hydroxy-9-octadecenoic acid)],
- mixed triglycerides having long- and short-chain moieties (such as diacetostearin), or in general,
- any materials containing constituents which have functional groups which give rise to specific absorption bands at or sufficiently close to interfere with the 966 cm^{-1} band of the C-H deformation of the isolated *trans* double bond.

NOTE Dienes, such as *cis-trans* and *trans-trans* dienes, can affect the calibration.

For accurate determinations on materials with *trans* levels below 5 %, a standardized capillary GLC method is recommended (for example, see ISO 15304).

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

ISO 5509, *Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
percentage of *trans* fatty acids
% trans
quantity of those substances in the sample, expressed in terms of the mass equivalent of methyl elaidate, divided by the sample mass expressed in grams per 10 ml of solvent

4 Principle

In most naturally occurring vegetable fats and oils, unsaturated constituents contain only isolated (i.e. non-conjugated) double bonds in the *cis* configuration. These *cis* bonds may be isomerized to the *trans* configuration during extraction and processing procedures, due to oxidation, conversion during heating, and/or partial hydrogenation. Animal and marine fats and oils may contain measurable amounts of naturally occurring *trans* isomers. Isolated *trans* bonds in long-chain fatty acids, fatty acid esters and triglycerides may be measured by infrared (IR) spectrometry. An absorption band with a maximum at 966 cm^{-1} (10,3 µm), arising from a C-H deformation about a *trans* double bond, is exhibited in the spectra of all compounds containing an isolated *trans* group. This band is not observed in the spectra of the corresponding *cis* and saturated compounds. Measurement of the intensity of this absorption band under analytically controlled conditions is the basis for a quantitative method for the determination of the isolated *trans* isomer content. For higher accuracy, common interfering absorptions associated with the glycerol backbone of triglycerides and the carboxyl groups of fatty acids must be eliminated by conversion of these samples to their methyl esters prior to analysis.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 Carbon disulfide (CS_2), dry.

WARNING — Prolonged breathing of carbon disulfide vapours is dangerous. This solvent should be handled only under conditions that provide adequate ventilation, preferably under a fume hood.

5.2 Primary standards: methyl elaidate and methyl oleate, with a purity of 99 %[1].

6 Apparatus

6.1 Infrared spectrometer (FT-IR or dispersive), capable of making measurements at 4 cm^{-1} resolution in the spectral range covering 1 050 cm^{-1} to 900 cm^{-1}.

The data-handling capability of the instrument should allow conversion of spectra to absorbance, scale expansion of the x- and y-axes, and readout of wave numbers to the nearest 1 cm^{-1} and of absorbance to the nearest 0,001 AU. FT-IR spectrometers should use TGS or DTGS detectors, or ensure linearity.

6.2 Infrared liquid sampling cells, with NaCl or KBr windows and a fixed optical path length of 1 mm.

For use in null-type instruments, pairs of cells matched to within 0,01 AU are required. In split-beam type instruments with both cells filled with the solvent (5.1), electronic balance of the two beams to within these limits should be attained.

6.3 Volumetric flasks, class A, of capacities 10 ml, 25 ml and 50 ml.

6.4 Pipettes, class A, of capacities 1 ml, 3 ml, 4 ml, 5 ml, 7 ml and 9 ml.

6.5 Disposable Pasteur pipettes, for filling infrared cells with the test sample.

6.6 Analytical balance, with a 60 g capacity, capable of weighing 0,2 g to an accuracy of ± 0,000 1 g.

---

[1] These are available from Nu-Check-Prep, Inc., Elysian, MN, USA. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

7 Sampling

Sampling is not part of the method given in this International Standard. A recommended sampling method is given in ISO 5555.

It is important that the laboratory receive a sample that is truly representative and has not been damaged or changed during transport and storage.

8 Preparation of test sample

Prepare the test samples in accordance with ISO 661.

Laboratory samples of solid fats shall be completely melted on a steam bath at a temperature not more than 10 °C above the melting point of the sample. The melted laboratory sample should be completely mixed before taking the test sample. Samples that appear cloudy because of the presence of water should be treated with anhydrous sodium sulfate and filtered before taking the test sample.

9 Procedure

9.1 Preparation of standards

9.1.1 Stocks solutions

Weigh, to the nearest 0,000 1 g, 0,5 g ± 0,01 g of methyl elaidate (5.2) into a 25 ml volumetric flask (6.3). Dilute to volume with solvent (5.1), stopper and mix well. Prepare a second solution of methyl oleate (5.2) in exactly the same manner. These stock solutions have a concentration of 0,020 g/ml. Prepare a 0,002 0 g/ml stock solution of methyl elaidate by pipetting 5,00 ml of the 0,020 g/ml methyl elaidate stock solution into a 50 ml volumetric flask (6.3). Dilute to volume with solvent (5.1), stopper and mix well.

9.1.2 1 %, 4 % and 7 % *trans* standards

Into 10 ml volumetric flasks (6.3), weigh, to the nearest 0,000 1 g, the mass of methyl oleate (5.2) specified in Table 1 to within ± 0,005 g. Pipette (6.4) the corresponding specified volume of the 0,002 g/ml methyl elaidate stock solution (9.1.1) into each of the 10 ml volumetric flasks. Dilute to volume with solvent (5.1), stopper and mix well.

Table 1 — Proportions for 1 %, 4 % and 7 % *trans* standards

Nominal *trans*-isomer content %	Methyl elaidate solution ml	Methyl oleate g
1	1,00	0,198
4	4,00	0,192
7	7,00	0,186

9.1.3 10 %, 30 %, 50 % and 70 % *trans* standards

Into 10 ml volumetric flasks (6.3), pipette the volume of the 0,020 g/ml methyl elaidate and methyl oleate stock solution (9.1.1) specified in Table 2. Confirm that the pipetted volumes accurately fill the flask up to the 10 ml graduation mark.

Table 2 — Proportions for 10 %, 30 %, 50 % and 70 % *trans* standards

Nominal *trans*-isomer content %	Methyl elaidate solution ml	Methyl oleate solution g
10	1,00	9,00
30	3,00	7,00
50	5,00	5,00
70	7,00	3,00

9.2 Calibration

9.2.1 General

For each standard (9.1.2 and 9.1.3), calculate the exact mass of methyl elaidate diluted in 10 ml of solvent.

Analyse each standard (9.1.2 and 9.1.3) and determine the baseline-corrected infrared absorbance at 966 cm^{-1} as described in 9.2.2 and 9.2.3.

9.2.2 For *trans* content $\leqslant$ 10 %

Using a first-order regression analysis, determine the slope and intercept of the line which best fits the plot of the baseline-corrected absorbance at 966 cm^{-1} for the 1 %, 4 %, 7 % and 10 % *trans* standards (y-axis) as a function of the grams of methyl elaidate per 10 ml of solution (x-axis).

9.2.3 For *trans* content $>$ 10 %

Repeat calibration step 9.2.2 for the 7 %, 10 %, 30 %, 50 % and 70 % *trans* standards.

9.3 Preparation of methyl esters

9.3.1 Convert a 0,5 g to 1,0 g portion of the sample (Clause 8) to methyl esters using the method given in ISO 5509, or any other accepted, standardized method that is appropriate for the type of sample (e.g. references [4], [5] or [6]).

9.3.2 Weigh, to the nearest 0,000 1 g, 0,20 g ± 0,01 g of the prepared (9.3.1), neat methyl esters into a 10 ml volumetric flask (6.3). Dilute to volume with solvent (5.1), stopper and mix well.

For oils and fats containing short-chain fatty acids, care should be taken in the preparation and concentration of the methyl esters to reduce the loss of the more volatile fractions.

9.4 Determination

9.4.1 General

Set up the operating parameters of the infrared spectrometer (6.1) according to the manufacturer's recommendations to collect infrared spectra at 4 cm^{-1} resolution covering the spectral range 1 050 cm^{-1} to 900 cm^{-1}. Conditions employed should be identical for both standards and samples.

Once calibration data have been established, they should be checked periodically to ensure their validity. For highest quantitative accuracy and precision, a primary or secondary reference should be methylated and analysed with each group of samples, then checked to ensure that the measured *trans* level is in agreement with the known or established mean value.

Infrared spectrometers should be checked to ensure that they are operating within the manufacturer's established specifications. Checks should include signal-to-noise as well as photometric and wavelength accuracy.

9.4.2 Single-beam instruments

Fill a clean infrared liquid cell (6.2) with solvent (5.1). Remove any air bubbles, stopper and place in the spectrometer cell holder. Record the single-beam spectrum to be used as a reference (background). Refill the clean cell with the solution obtained in 9.3.2 and record the single-beam spectrum of the sample. Ratio the sample spectrum against the background and convert to absorbance units.

9.4.3 Split- or dual-beam instruments

Fill a clean infrared liquid cell (6.2) with solvent (5.1). Remove any air bubbles, stopper and place in the reference beam cell holder. Fill a second, matched cell (6.2) with the solution obtained in 9.3.2. Remove any air bubbles, stopper and place in the sample beam cell holder. Record the spectrum and convert to absorbance units.

10 Expression of results

10.1 With the spectrum of the sample scale expanded in the region from 1 050 cm^{-1} to 900 cm^{-1}, draw a baseline (XY) tangent to the “feet” of the infrared absorbance band at 966 cm^{-1} as demonstrated in Figure 1. The points in a spectrum between which line XY is drawn will vary with peak absorbance. For accurate results, baselines shall be drawn the same for both standards and samples. For samples with high *trans* content, the baseline should be drawn to the minimum located at 985 cm^{-1}. Occasionally, this minimum may be absent and the point to which the baseline is drawn shall be estimated.

10.2 Determine the baseline-corrected absorbance of the band (A_C) by subtracting the absorbance of the baseline at the peak maximum (A_B) from the absorbance of the peak at 966 cm^{-1} (A_P). The measured position of the peak maximum will vary with instrument and the *trans* content of the sample analysed. The position of the maximum should be established separately for each instrument with the 70 % *trans* standard. This same position should then be used at all concentrations.

10.3 From Figure 1

$$A_\text{C} = (A_\text{P} - A_\text{B})$$

Using the calibration data for the appropriate *trans* range ($\leqslant$ 10 % or $>$ 10 %), calculate the mass equivalent of methyl elaidate, m_equiv, in grams, in the sample (9.3.2) from the following equations:

$$m_\text{equiv} = \frac{A_\text{C} - a}{b}$$

$$\omega_\text{trans} = \frac{m_\text{equiv}}{m_\text{t}} \times 100\ \%$$

where

A_C is the baseline-corrected absorbance of the band;

a is the intercept;

b is the slope;

ω_trans is the % *trans* as methyl elaidate;

m_t is the mass of sample per 100 g of solvent.

10.4 Report results to the nearest 0,1 %.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than the repeatability limit r as shown in Annex A.

11.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the reproducibility limit R as shown in Annex A.

12 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result obtained; or

f) if the repeatability has been checked, the final quoted result obtained.

© ESA

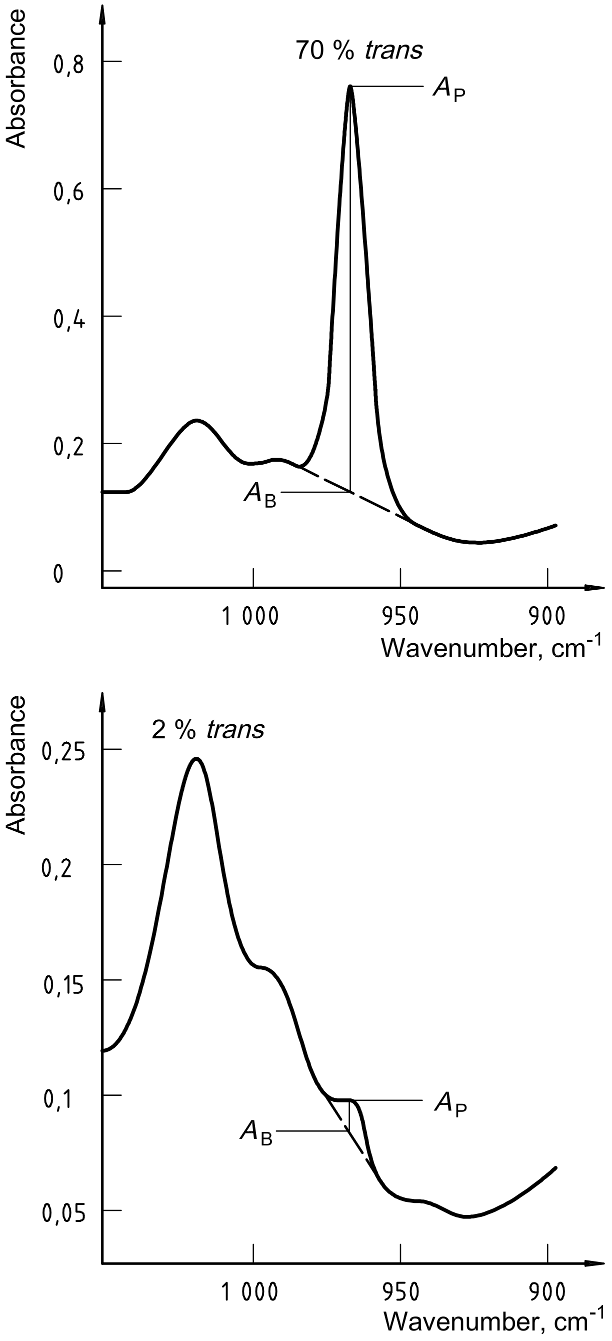

Figure 1 — Infrared spectra of methyl esters containing 70 % and 2 % of the *trans* isomer

Annex A
(informative)

Results of interlaboratory test

An international interlaboratory test, completed by the AOCS Chromatography Committee in 1995, in which 16 laboratories participated, each obtaining two test results for each sample analysed by IR, gave statistical results determined in accordance with ISO 5725-1, which are summarized in Table A.1.

Table A.1 — Statistical results

	Sample [a]						
	Me-1	**Tg-1**	**Tg-2**	**Tg-3**	**Tg-4**	**Tg-5**	**Tg-6**
Number of participating laboratories	16	16	16	16	16	16	16
Number of laboratories retained after eliminating outliers	15	16	16	15	16	16	14
Number of individual test results of all laboratories on each sample	30	32	32	30	32	32	27
Mean value, % *trans*	20,23	6,09	1,33	16,14	16,02	30,91	47,25
Known value, % *trans*	21,15	6,14					
Repeatability standard deviation (s_r)	0,49	0,22	0,18	0,20	0,38	0,46	0,81
Repeatability coefficient of variation, %	2,40	3,65	13,86	1,21	2,38	1,49	1,71
Repeatability limit (r)	1,37	0,62	0,50	0,56	1,09	1,29	2,27
Reproducibility standard deviation (s_R)	1,30	0,60	0,44	1,19	1,07	1,86	1,22
Reproducibility coefficient of variation, %	6,42	9,82	32,78	7,38	6,69	6,03	2,59
Reproducibility limit (R)	3,64	1,68	1,23	3,33	3,00	5,21	3,42

[a] Me-1 = methyl elaidate/methyl stearate with 21,15 % *trans*;
Tg-1 = trielaidin with 6,14 % *trans* in zero-*trans* peanut oil;
Tg-2 = canola oil (rapeseed oil);
Tg-3 = shortening;
Tg-4 = shortening;
Tg-5 = margarine;
Tg-6 = frying oil.

NOTE Tg-3 and Tg-4 were blind duplicates.

Bibliography

[1] ISO 5555:2001, *Animal and vegetable fats and oils — Sampling*

[2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 15304:2002, *Animal and vegetable fats and oils — Determination of the content of* trans *fatty acid isomers of vegetable fats and oils — Gas chromatographic method*

[4] AOCS Official Method Ce 2-66, *Preparation of Methyl Esters of Long-chain Fatty Acids*

[5] AOAC Official Method 969.33, *Fatty Acids in Oils and Fats — Preparation of Methyl Esters — Boron Trifluoride Method*

[6] IUPAC 2.302, *Preparation of the fatty acid methyl esters*

የኢትዮጵያ የደረጃዎች ኤጀንሲ
Ethiopian Standards Agency

DRAFT

Organization and Objectives

The Ethiopian Standards Agency (ESA) is the national standards body of Ethiopia established in 2010 based on regulation No. 193/2010.ESA is established due to the restructuring of Quality and Standards Authority of Ethiopia (QSAE) which was established in 1970.

ESA's objectives are:-

- Develop Ethiopian standards and establish a system that enable to check weather goods and services are in compliance with the required standards,
- Facilitate the country's technology transfer through the use of standards,
- Develop national standards for local products and services so as to make them competitive in the international market.

Ethiopian Standards

The Ethiopian Standards are developed by national technical committees which are composed of different stakeholders consisting of educational Institutions, research institutes, government organizations, certification, inspection, and testing organizations, regulatory bodies, consumer association etc. The requirements and/ or recommendations contained in Ethiopian Standards are consensus based that reflects the interest of the TC representatives and also of comments received from the public and other sources. Ethiopian Standards are approved by the National Standardization Council and are kept under continuous review after publication and updated regularly to take account of latest scientific and technological changes.
Orders for all Ethiopian Standards, International Standard and ASTM standards, including electronic versions, should be addressed to the Documentation and Publication Team at the Head office and Branch (Liaisons) offices. A catalogue of Ethiopian Standards is also available freely and can be accessed in from our website.

ESA has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of ESA.

International Involvement

ESA, representing Ethiopia, is a member of the International Organization for Standardization (ISO), and Codex Alimentarius Commission (CODEX). It also maintains close working relations with the international Electro-technical Commission (IEC) and American Society for Testing and Materials (ASTM).It is a founding member of the African Regional Organization for standardization (ARSO).

More Information?
Contact us at the following address.
The Head Office of ESA is at Addis Ababa.

☎ 011- 646 06 85, 011- 646 05 65
📠 011-646 08 80
✉ 2310 Addis Ababa, Ethiopia
E-mail: info@ethiostandards.org,
Website: www.ethiostandards.org

Standard Mark